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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
CG/12326.19		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA99/00852	15/09/1999	15/09/1998
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant SIGNALGENE INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06/04/2000	22.12.2000
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Form PCT/IPEA/409 (cover sheet) (January 1994)

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WHAT IS CLAIMED IS:

1. A method of determining an individual's predisposition to breast cancer, development of breast cancer and/or responsiveness to therapy for breast cancer, said method comprising the step of determining a polymorphism at the CAG repeat of the androgen receptor (AR) gene or a DNA variant equivalent, or mutation which shows a linkage disequilibrium therewith, whereby said polymorphism at the AR gene, or marker in linkage disequilibrium therewith enables a prediction of an individual's predisposition to breast cancer, development of breast cancer and/or responsiveness to therapy for breast cancer.

2. The method of claim 1, wherein the androgen receptor genotype is determined by determining the number of CAG repeats within the androgen receptor gene

3. The method of claim 2, which further comprises a step of amplifying a segment of the androgen receptor using polymerase chain reaction.

4. The method of claim 3, wherein a pair of primers derived from a nucleic acid sequence of the androgen receptor gene or flanking said gene is used in the polymerase chain reaction.

5. The method of claim 4, wherein the segment of the androgen receptor gene is amplified using a pair of primers as follows:

5'-TCCAGAATCT GTTCCAGAGC GTGC-3' SEQ ID NO:1; and
5'-GCTGTGAAGG TTGCTGTTCC TCAT-3' SEQ ID NO:2.

6. The method according to one of claims 1 to 5, wherein said polymorphism at the AR gene, or marker in linkage disequilibrium therewith, is determined from DNA obtained from said individual.

7. The method of claim 6, wherein said DNA is genomic DNA.

8. The method according to claim 7, wherein said DNA is obtained from non-cancerous cells.

9. The method of claim 8, wherein said cell is obtained from a tissue or blood sample.

10. An assay for screening and selecting an agent which modulates breast cancer predisposition comprising:

a) a recombinant androgen receptor (AR) gene or functional fragment thereof, which comprises a CAG repeat polymorphism in exon 1 thereof, or a marker in linkage disequilibrium therewith; and

b) assaying a function of said androgen receptor;

wherein an allele which modulates said function of said androgen receptor can be selected, and wherein a modulation of a function of said androgen receptor is associated with a modulation of said breast cancer predisposition, whereby short CAG repeats of said AR positively modulate androgen receptor function, while long CAG repeats of said AR negatively modulate Androgen receptor function, thereby leading to breast cancer protection or breast cancer predisposition.

11. An assay for screening and selecting an agent which modulates breast cancer predisposition comprising:

a) an expression vector comprising a promoter operably linked to a reporter gene, said promoter comprising an androgen response

element, said response element affecting the activity of said promoter upon binding thereto of androgen or analog thereof;

b) a cell expressing a chosen allele of an androgen receptor and harboring said vector of a);

c) submitting said cell to at least one agent; and

d) assaying a level of said reporter gene;

whereby an agent which modulates breast cancer predisposition can be selected when the level of said reporter gene is significantly modulated by the presence of said agent through its action through the androgen receptor.

12 A method for screening and selecting an agent which can modulate breast cancer predisposition comprising:

a) selecting a specific allele of the androgen receptor (AR) gene, variant, equivalent, or mutation thereof which shows linkage disequilibrium therewith;

b) assaying a function of said AR allele of a); and

c) selecting an agent which can modulate breast cancer predisposition,

wherein an agent which modulates AR function is selected as an agent capable of modulating breast cancer predisposition when said function is significantly different in the presence of said agent, as compared to in the absence thereof.

13. The method of one of claims 1 to 9, or 12, wherein the shortest alleles or a combination thereof are associated with a protection to breast cancer, and the intermediate to large alleles or a combination of the intermediate and largest alleles are associated with a predisposition to breast cancer.

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Breast cancers have the same clinical characteristics in older as in younger women. Cancer is usually suspected when changes are noted on mammography or when a breast lesion is seen or felt. Lesions usually can be felt as firm nodules within the breast. Ulcerations may occur, and lesions within or near the nipple may produce discharge. Sometimes breast cancer is discovered only after metastatic lesions cause bone fractures, neurologic changes, hypercalcemia, liver failure, or ascites.

When a tumor is detected by physical examination, bilateral mammograms are normally obtained to rule out occult lesions. Certain radiographic images, such as speckled calcifications or tissue infiltration, suggest cancer, while a cystic appearance suggests a benign process. Even an apparently benign finding on mammogram requires further evaluation. Generally the diagnosis is established by fine needle aspiration. Fine needle aspiration allows collection and cytological examination of cystic fluid and is helpful in planning definitive treatment of breast cancer. Although a positive result on fine needle aspiration is diagnostic, a negative result is usually followed by an open biopsy. Now a day, there is still no specific test for assaying predisposition or resistance to breast cancer.

Since the discovery of the human androgen receptor (AR) gene, mutations in this gene have been associated with Kennedy's disease (spinobulbarmuscolar atrophy), with various degrees of androgen insensitivity and with prostate cancer.

Elhaji et al. (American Journal of Human Genetics, vol. 61, no. 4 suppl, 28.10.1997 – 1.11.1997, page A64) assesses the distribution of CAG-repeat length of the AR in breast cancer tissue to evaluate the possible correlation between the repeat length and the risk of breast cancer. However, Elhaji et al. is concerned with breast cancer tissue *per se* and not with the potential of using AR as a marker for determining the predisposition and prognosis of breast cancer by, for example, screening patients prior to the

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development of breast cancer. Indeed, Elhaji et al. suggests that somatic mutations are involved in shifting the distribution of the CAG repeat of the AR gene and thus, that a predisposition and prognosis test could not be carried-out.

Thus, an association between a germline mutation in AR gene and predisposition to breast cancer has yet to be reported.

There thus remains a need to provide a genetic assay for determining the predisposition and/or resistance to breast cancer, development of breast cancer and responsiveness to therapeutic modalities.

While some markers have been identified as genetic determinants for breast cancer and/or as risk factors to develop same (i.e. BRCA1 and BRCA2), there remains a need to identify new markers therefor. More specifically, there remains a need to provide means to determine a